

## EFFECT OF ESSENTIAL OILS OF SELECTED SPICES IN FOOD SYSTEMS

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### ABSTRACT

Essential oils (EOs) of spices and herbs have substantial anti-microbial effects. Most of them have the GRAS (*Generally Regarded as Safe*) and FA (*food additive*) status by the FDA (Food and Drug Administration, USA) and can be used in foodstuffs as natural preservatives. However, the complex system of foods can protect spoilage microorganisms from the anti-microbial effect of essential oils. Our aim was to observe the germ count reducing effect of certain essential oils (cinnamon, lemon, marjoram and thyme oil) on selected bacteria and yeasts (*Bacillus cereus*, *Escherichia coli*, *Geotrichum candidum* and *Saccharomyces cerevisiae*) in growth medium, as well as in different foods and beverages (apple juice, milk, milk rice and minced pork meat). Our results showed that bacteria and yeasts are less sensitive to the EOs in foods than in growth medium. The minimal inhibitory concentrations (MICs) in liquid media varied from 0.125 µl/ml to >2 µl/ml. The MIC for lemon oil on *S. cerevisiae* in apple juice was 1 µl/ml and on *G. candidum* in skimmed milk was >4 µl/ml. One percent cinnamon or marjoram oil was required to achieve a 1 log cell count reduction of *B. cereus* in milk rice or *E. coli* in minced pork. These EO concentrations resulted in a strong odor of the foods making them hardly consumable. In the future, the combination of EOs with other preservatives or with hurdle techniques can eliminate the problems arising from these unfavorable strong aromas.

### 1. INTRODUCTION

Essential oils (EOs) in aromatic plants are among the most important active constituents of herbs and spices. Their efficiency against a wide range of microorganisms is well documented. There is a growing interest in using EOs as natural preservatives against food spoilage- and food-borne pathogen microbes, in order to meet consumer demands for avoiding synthetic components in food (Bagamboula et al., 2003). It has been often observed that the preservative effect in food can be achieved by higher doses of EOs than *in vitro* (Burt, 2004). It is supposed that foodstuffs with high protein and fat content can protect bacteria from the antibacterial effect of EOs (Gill et al., 2002; Smith-Palmer et al., 2001). An essential oil dissolved in the fat of the food will be less available to act on bacteria present in the aqueous phase.

In our experiments the antibacterial and antifungal activities of cinnamon, lemon, marjoram and thyme essential oils was investigated against the bacteria *Bacillus cereus*, and *Escherichia coli*, and the yeasts *Saccharomyces cerevisiae* and *Geotrichum candidum*.

*B. cereus* is a Gram positive, facultative anaerobe, endospore forming bacterium. The endospores can survive in improperly cooked foods, especially in rice, causing food poisoning through the production of enterotoxins.

*E. coli* is a Gram negative bacterium living in the gut of mammals. Most strains are harmless but some serotypes can cause serious food poisoning in humans. *E. coli* is an indicator bacterium for fecal contamination of foods through poor personal or slaughter hygiene.

Spoilage yeasts cause the deterioration of plant-derived products, especially fruit and vegetable juices. *Saccharomyces cerevisiae* causes ethanol fermentation in "open" beverages. Yeast can also be found in dairy products such as yoghurt, kefir, and soft and fresh cheeses (Deák, 2007). The presence of *Geotrichum candidum* is a common problem in raw milk used for the production of soft cheeses and other dairy products.

Our aim was to evaluate the antibacterial and anti-yeast action of the essential oils in real food systems, such as apple juice, milk rice and ground meat.

## 2. MATERIALS AND METHODS

### 2.1. Essential oils (EOs)

The essential oils (EOs) investigated in this study were thyme, marjoram, lemon and cinnamon oil. The EOs were provided from Aromax Natural Products (Budapest, Hungary).

### 2.2. Bacteria and culture conditions

*B. cereus* var. *mycoides* ATCC 9634 was cultured on meat extract medium (MEE; 0.4% meat extract, 0.4% peptone, 1% glucose, 0.1% yeast extract), *Escherichia coli* SZMC 0582 was grown on Luria-Bertani medium (LB; 1% tryptone, 0.5% yeast extract, 1% NaCl). *B. cereus* was incubated at 30 °C, and *E. coli*, at 37 °C.

### 2.3. Yeasts and culture conditions

The yeasts were isolated from spoiled food sources. *Geotrichum candidum* MB-102 was isolated from spoiled cottage cheese, and *S. cerevisiae* MB-21 from wild-growing black currants. Yeasts were grown on malt extract medium (ME; 0.4% malt extract, 1% glucose, 0.1% yeast extract) at 30 °C. Isolates were maintained on ME agar slants at 4 °C.

### 2.4. Determination of Minimal Inhibitory Concentration (MIC) values in growth media and in food systems

The MICs of the EOs were determined by macro dilution assay. 100 µl from the stock solutions of the EOs made in 50 % DMSO were added to 5 ml growth medium or apple juice resulting in final concentrations of from 0.0625 µl/ml to 2 µl/ml in twofold increments. The tubes were inoculated with approximately 10<sup>5</sup> CFU/ml bacterium or yeast and then incubated at 30 °C or 37 °C for 24 h. MICs were taken as the lowest concentration at which no visible growth occurred.

MIC values in milk were evaluated by plate count after 24 h incubation at 30 °C. The EO concentration where no colony formation was observed was taken as the MIC.

### 2.5. Antibacterial activity in minced pork and in milk rice:

Essential oils of thyme and marjoram were added to the minced pork, previously inoculated with 2 x 10<sup>5</sup> *E. coli*, in a concentration range from 0.125 to 1% (w/w). After 24



h storage at 4 °C, *E. coli* CFU (Colony Forming Unit) of the meat was determined on VRBG agar (Merck).

Cinnamon oil was added to fresh cooked and to room temperature cooled milk rice in the concentration range from 0.125% to 1 % (w/w). The rice was inoculated with 10<sup>5</sup> CFU/ml *B. cereus*. After 24 h storage at 4 °C bacterial CFU was determined on TGE agar.

### 3. RESULTS AND DISCUSSION

#### 3.1. Minimal Inhibitory Concentration (MIC) values

The minimal inhibitory concentrations can be seen in Table 1. In growth medium marjoram had the lowest MIC value on *E. coli* with 0.125 µl/ml. In beverages the MIC was higher than in the medium. In minced pork and in milk rice we were not able to determine the MIC in the used concentration range.

Table 1: MIC values (µl/ml) of the investigated oils in growth medium (M) and in food systems (F). *F<sub>mm</sub>* – minced meat; *F<sub>aj</sub>* – apple juice; *F<sub>m</sub>* – semi skimmed milk, *F<sub>mr</sub>* – milk rice

EO	Microorganisms							
	<i>E. coli</i>		<i>S. cerevisiae</i>		<i>G. candidum</i>		<i>B. cereus</i>	
	M	<i>F<sub>mm</sub></i>	M	<i>F<sub>aj</sub></i>	M	<i>F<sub>m</sub></i>	M	<i>F<sub>mr</sub></i>
Cinnamon	-	-	-	-	-	-	1	n.d.
Lemon	-	-	0.5	1	1	>4	-	-
Marjoram	0.125	n.d.	-	-	-	-	-	-
Thyme	>2	n.d.	-	-	-	-	-	-

n.d. not determined

#### 3.2. Growth inhibition effect of essential oils in real foods

In minced meat and in milk rice the CFU count of the inoculated bacteria do not reach zero under the given experimental conditions. We have monitored a cell number reduction on higher concentrations of the EOs. The maximal CFU reduction (1.8 log) was reached in the presence of 1 % cinnamon oil in milk rice. In minced pork marjoram EO showed better germ reduction capacity with 0.74 log than thyme oil with 0.5 log. Results are presented in Table 2.

Table 2: CFU count of the infection bacteria in minced pork (*E. coli*) and in milk rice (*B. cereus*) after 24 h storage at 4 °C.

Essential oil concentration (%)	Microorganism		
	<i>E. coli</i>		<i>B. cereus</i>
	Marjoram	Thyme	Cinnamon
0	4.1 x 10 <sup>5</sup>	4.1 x 10 <sup>5</sup>	7.6 x 10 <sup>4</sup>
0.25	6.5 x 10 <sup>5</sup>	7.5 x 10 <sup>5</sup>	4.8 x 10 <sup>4</sup>
0.5	3 x 10 <sup>5</sup>	2.2 x 10 <sup>5</sup>	1.3 x 10 <sup>4</sup>
1	7.5 x 10 <sup>4</sup>	1.3 x 10 <sup>5</sup>	1.2 x 10 <sup>3</sup>

There are some examples in the literature on the application of essential oils in meat or fruit based foods. In the study by Busatta et al (2008), MIC of marjoram essential oil in

growth medium against *E. coli* was 0.92 mg/ml. They applied marjoram oil in fresh sausage inoculated with *E. coli*, and after 25 days of incubation the MPN (most probably number) was reduced by approximately 1 log. The main component in marjoram EO having antibacterial activity is terpinene-4-ol, causing changes in membrane permeability. The main component of thyme essential oil, thymol, a phenolic constituent, was effective on coliforms and *Enterobacteriaceae* in minced beef patties (Del Nobile et al. 2009). In our experiments, thyme EO had only a slight CFU reduction effect which might be due to differences in its composition.

Cinnamon or citrus-based EOs were used to improve the shelf-life of fruit- or milk-based acidic foods or beverages (Smith-Palmer et al., 2001; Belletti et al., 2007). Citrus EOs have been successfully used against food spoilage yeasts (Viuda-Martos et al., 2003; Belletti et al., 2007). Aroma compounds and citron EO combined with mild heat treatment inhibited the growth of *S. cerevisiae* in non-carbonated soft drinks (Belletti et al., 2007).

The increased amount of essential oils in foods necessary to achieve good antimicrobial effect has a negative impact on the organoleptic properties of foods. The 1% concentration of marjoram and thyme EO in minced pork caused a very strong smell making the meat hardly consumable.

The combination of EOs with other preservatives or with different hurdle technologies can reduce the amount of EOs required and can eliminate the problems relating to their strong aroma (Pol et al., 2001; Gill et al., 2002; Atrea et al., 2009; de Souza et al., 2009).

#### 4. CONCLUSION

Essential oils had better efficacy against spoilage microbes in beverages with high carbohydrate content than in foods with high protein and/or fat content. Further work is needed to determine the smallest effective concentration of EOs in foods, especially in beverages, to achieve good antimicrobial effect but avoid serious changes in taste and odour.

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